Title: A method for the objective selection of landscape-scale study sites at the national level

Running title: Landscape site selection

Authors: Mark A. K. Gillespie[1,2], Mathilde Baude[3,4], Jacobus Biesmeijer[5], Nigel Boatman[6], Giles E Budge[6,7], Andrew Crowe[6], Jane Memmott[3], R. Daniel Morton[8], Stephane Pietravalle[6], Simon G. Potts[9], Simon Smart[8], William E. Kunin[1]

Affiliations

[1]School of Biology, University of Leeds, Leeds LS2 9JT, UK

[2]Department of Science & Engineering, Sogn & Fjordane University College, PB 133, 6851 Sogndal, Norway

[3]School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG UK

[4] Collegium Sciences et Techniques EA 1207 LBLGC, Université d'Orléans, F-45067, Orléans, France

[5]Naturalis Biodiversity Center, 2333 CR Leiden, the Netherlands & Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, the Netherlands

[6]Fera Science Ltd (previously Food and Environment Research Agency), Sand Hutton, York, YO41 1LZ UK

[7]Institute for Agri-Food Research and Innovation, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK.

[8]Centre for Ecology & Hydrology, Lancaster LA1 4AP, UK

[9]Centre for Agri-Environmental Research, School of Agriculture, Policy and Development, University of Reading, Reading RG6 6AR, UK

Corresponding author: Mark Gillespie, Department of Science & Engineering, Sogn & Fjordane University College, PB 133, 6851 Sogndal, Norway, mark.gillespie@hisf.no

Keywords: Experimental design, Site selection, Remote sensing, Insecticides, Floral resources, Habitat diversity, Honey bees, Pollinators, accidental experiments, natural experiments

Word count: 6541

Abstract

1) The impact of land use on populations, communities and ecosystem functions is an important area of research, but ecological processes operating on large spatio-temporal scales are difficult to disentangle with traditional empirical approaches. Alternatively, researchers can take advantage of “natural” experiments, where control is solely exercised by site selection. However, to use this approach, unbiased and objective site selection protocols are needed. Here we detail a unique, objective large-scale site selection protocol, developed to study the impact of multiple landscape scale factors on pollinator abundance and diversity across Britain.
2) Using datasets of geographic and ecological variables with national coverage, we applied a novel hierarchical computation approach to select study sites that contrast as much as possible in four key variables, while attempting to maintain regional comparability and national representativeness. There were three main steps to the protocol: i) selection of six 100 km x 100 km regions that collectively provided land cover representative of the national land average, ii) mapping of potential sites into a multivariate space with axes representing four key factors potentially influencing insect pollinator abundance, and iii) applying a selection algorithm which maximised differences between the four key variables, while controlling for a set of external constraints.
3) Validation data for the site selection metrics were recorded alongside the collection of data on pollinator populations during two field campaigns. While the accuracy of the metric estimates varied, the site selection succeeded in objectively identifying field sites that differed significantly in values for the four key variables. Between variable correlations were also reduced or eliminated, thus facilitating analysis of their separate effects.

4) This study has shown that national datasets can be used to objectively select randomised and replicated field sites along multiple interacting gradients. The network of field sites identified by this protocol could be used as the basis for studying a range of alternative research questions related to land use or other spatially explicit environmental variables, and this protocol could be replicated to identify networks of field sites for other countries, regions, drivers, and response taxa in a wide range of scenarios.

Introduction

With almost 40% of the world's land surface currently converted to human use (Ramankutty *et al.* 2008; Tanentzap *et al.* 2015), the impact of land use and habitat cover on biodiversity and ecosystem functions are key research themes in ecology, particularly for landscape ecologists (Wu & Hobbs 2002; Cumming *et al.* 2013). However, a major challenge facing researchers of large-scale processes is to find appropriate methods to characterise relationships between land use and biodiversity patterns (Diamond 1983; Hargrove & Pickering 1992; Bowers & Dooley 1999; Wu & Hobbs 2002; Mitchell *et al.* 2006; Dilts, Yang & Weisberg 2010; Smart *et al.* 2012; HilleRisLambers *et al.* 2013). This study aims to address this challenge by detailing a novel protocol for objectively selecting large-scale study sites to better test the links between focal variables and biodiversity patterns.

At the landscape or regional context it is extremely difficult to apply a classical experimental approach by establishing controls, manipulating "treatments", assigning large-scale experimental units to treatments randomly or achieving true replication (Hargrove & Pickering 1992; Hobbs 2003; Rundlof *et al.* 2015). In response to these issues, landscape ecology as a discipline has developed a number of tools to study large-scale natural phenomena, with much written about the value of observational research methods when the sampling approach is carefully designed (Diamond 1983; Hargrove & Pickering 1992; Sagarin & Pauchard 2010; HilleRisLambers *et al.* 2013). Many landscape-scale observational studies take place within "natural" or "accidental experiments", making use of existing environmental variation occurring due to some sudden event or the gradual change brought about by humans or nature or both. In the absence of plot manipulation on a massive scale, experimental control is carried out through the selection of sites (Diamond 1983). However, the researcher may not always be able to control, measure, or even identify all the important factors in the study (Diamond 1983; Stow *et al.* 1998; Smart *et al.* 2012). In addition, researchers may introduce potential biases, and problems of repeatability and generality of results by only studying well-known landscapes close to the study base or research institution (Dilts, Yang & Weisberg 2010). Therefore, better methods are needed for objectively selecting study sites in landscape ecology projects, in order to ensure more generality of results.

By choosing study sites more objectively and without bias, cross-site comparisons can be used to reveal the relative importance of different landscape-scale variables for focal populations and communities (Hargrove & Pickering 1992; Dilts, Yang & Weisberg 2010; Roux *et al.* 2013). To date, large-scale studies have either not attempted to cross, replicate and randomise gradients of hypothesised drivers or have optimised survey design for single drivers while attempting to hold other factors constant. While valuable, these studies do not provide the quantitative understanding needed to understand consequences of interacting drivers at realistic scales. A new method of site selection is therefore required to help disentangle multiple interacting drivers at large-scales.

This paper reports the development and testing of an objective site selection protocol as the basis for natural experiments at a national scale. The method was originally developed to study the links between land use / management variables and insect pollinator populations and communities, but the approach is generic and could be used at a range of spatial scales and applied to almost any taxa or system. The objectives of the site selection methodology were to improve on previous landscape-scale natural experimental designs by: i) enhancing objectivity (i.e., using a systematic approach with a transparent methodology which could be readily reproduced by other researchers), ii) enabling the study of several key factors simultaneously, and interactions between them, by selecting sites contrasting along multiple axes, and iii) enhancing the generality of results by selecting sites that are representative of an entire country. To do this, national datasets were used to first select a set of focal regions that would be representative of Britain, and then to characterise each potential field site within those regions in terms of four key landscape-scale metrics that are thought to affect insect pollinator populations (habitat diversity, floral resource availability, insecticide loadings, managed honey bee density). Field sites were chosen to contrast as much as possible in each of the four key metrics while attempting to maintain regional comparability and representativeness. Verification of the protocol was conducted by validating the values of the four metrics through in-situ surveys. The data demonstrate that landscape scale variation can be estimated using available national datasets, and thus suggest that similar approaches may be effective in addressing other large-scale issues.

Methods

The site selection protocol consists of three parts: 1) focal region selection, 2) site selection, and 3) testing of the site selection. These aspects are outlined below with full details given in the Supplementary material.

Focal Regions

To simplify field logistics and costs by limiting the amount of travel between sites, it was decided to first select six representative "focal regions" of 100 x 100 km, and then choose study landscapes within them. The regions were selected to maximise characteristics of the British landscape across vegetation and environmental gradients and the number of regions was chosen due to the time and financial resources available, but the protocol could easily be applied for a different number of regions. The selection of focal regions began with two 100 km resolution grids: the standard UK Ordnance Survey grid at 100 km resolution, and a second grid diagonally offset by 50 km to the east and north. All possible six-region combinations which did not include adjacent or overlapping cells were examined. For each six-region combination, the area in each broad habitat (from the 2007 Land Cover Map (LCM2007); Morton *et al.* 2011), and ITE Land Class (Bunce *et al.* 1996) were summed. LCM2007 broad habitats represent vegetation and, indirectly, land management; land classes represent topography, climate and human infrastructure. The Euclidean distance (i.e., the distance between the co-ordinates of two points on a graph) was calculated between each such mixture and the overall proportional contribution of these broad habitats or land classes in Britain as a whole. The combination that minimises this difference maximises the representativeness of the British landscape as a whole with respect to these variables, and was chosen as the set of focal regions to be studied.

Survey sites

The aim of the survey site selection protocol was to identify sites that contrasted as much as possible in four landscape-scale metrics: 1) habitat diversity, 2) floral resource availability, 3) insecticide loadings and 4) managed honey bee density. These four metrics were chosen because previous studies demonstrated that they are important drivers of local pollinator population decline in the UK. Strong links have been made between pollinator populations and the complexity of the landscape (Shackelford *et al.* 2013), the diversity and density of floral resources in agricultural settings (Potts *et al.* 2003; Gabriel & Tscharntke 2007) and increased insecticide usage (Rortais *et al.* 2005; Brittain *et al.* 2010). There is evidence that managed stocks of honey bees can affect the condition of wild pollinator stocks either through spill-over of parasites (e.g., Evison *et al*. 2012) or through competitive interactions

(Goulson & Sparrow 2009; Elbgami *et al.* 2014), although the landscape-scale population impact of honey bees on wild pollinators remains untested. The way in which these factors interact to impact pollinator populations is not fully understood, however (Vanbergen *et al.* 2013). In order to study the effects of these four factors individually and in combination, a total of 16 sites in each study region were sought, representing every combination of "high" versus "low" values of each metric. As the four metrics outlined above could be correlated (e.g. commercial beekeepers tend to place their hives close to dense floral resources), we used a computer algorithm technique to select sites with extreme values of each metric, as outlined below and in Supplementary material S1.1

Data sources and manipulation

Datasets were compiled using the UK Ordnance Survey National Grid reference system, the system of geographic grid references in the UK. The finest scale at which most agricultural and biodiversity datasets are available is the "tetrad" scale (2 x 2 km). Given the relatively high mobility of many pollinating insects (Westphal, Steffan-Dewenter & Tscharntke 2006), we opted to define our sites at this scale. For each of the 2,500 potential sites or tetrads within a 100 x 100 km region, a value for each of the metrics was calculated from national datasets. Full details of the calculations are given in Appendix S1.2, but they are briefly outlined here:

1) **Habitat diversity** was calculated as a Shannon diversity index of broad habitats present, with each weighted by the area covered within each candidate tetrad. Habitat areas were derived from the LCM2007 (Morten et al. 2011).
2) **Floral resource availability** was calculated from nectar data only as pollen data are less well recorded for British plants. This variable is expressed in terms of kilograms of sugar per hectare per year, and was derived by a) estimating flowering plant

species cover per unit area of each habitat type in each site using regional data from Countryside Survey 2007 (CS2007; Carey *et al.* 2008) and LCM 2007, b) modelling nectar sugar values for the 220 commonest insect-pollinated species based on published values for 124 species at the time of the study (see Table S2 for details and references), c) accounting for additional floral resources in mass-flowering crops, agri-environment schemes and in organic arable fields.

3) **Agricultural chemical loadings** were calculated by multiplying the area under cultivation of each of 36 crop groups within the sites estimated from national agricultural statistics, by a regional hazard score for that crop group, derived from Pesticide Usage Survey data for each crop combined with honey bee toxicity data for each insecticide applied.
4) **Managed honey bee population density** was estimated from data held by the national "Beebase" database (www.nationalbeeunit.com). The number of adult bees present in mid-summer for an average colony was estimated and this was combined with the typical number of colonies present in each of three apiary classes. Honey bee density in surrounding landscapes was modelled by using published honey bee foraging data (Waddington *et al.* 1994; Beekman & Ratnieks 2000). The apiary location was used as a centroid and the estimated number of honey bee foragers grouped into concentric 200 m bins (see Supplementary material).

Site selection algorithm

Once assigned, the metric values were standardised by a Box-Cox transformation and converted to z scores (zero-centred), so that a score below 0 for a metric corresponded to a low value relative to regional norms, and a score above 0 represented a high value. The objective of the algorithm was to select a combination of 16 sites within a 100 x 100 km focal region to emphasise contrast across the four metrics. The number of ways of drawing unique sets of 16 sites from the 2,500 options in a focal region is enormous ($1.06055 * 10^{41}$ combinations). It was therefore essential to reduce computing time by constraining the site combinations using a series of design criteria. These criteria included removing the sites closest to the mean value for any of the four variables, restricting the distance between sites to 50 km (for logistical reasons), restricting the amount of urban and water cover allowed per site, and ensuring topographic comparability between sites (e.g., to avoid sites on mountain tops vs valley floors). See Supplementary material S1.1.2 for full details of the selection criteria. Once a feasible combination of field sites had been selected, landowners were identified and contacted for access permission. If access permission was refused to more than 30% of the site, the next feasible combination of field sites was chosen.

Site selection: validation

As the four metrics were all assessed indirectly with varying degrees of reliability, their values were validated during a two-year field campaign. The full details of the validation processes are given in Supplementary material S1.2 but are outlined briefly here:

1) **Habitat diversity** values were validated by field surveys confirming or correcting the habitat types as mapped in the LCM2007. Corrected habitat areas were then used in new diversity index calculations.
2) **Floral resource availability.** Validation for this metric required several stages: a) actual floral reward production per flower per day was sampled for 175 species, and remodelled for a further 62 (2012) and 86 (2013) species (Baude *et al.* 2016), b) transect surveys were conducted to assess actual floral cover of each species for each broad habitat within each site, c) data from 1 and 2 were combined with corrected

habitat areas and corrected agri-environment scheme, mass-flowering crop and organic areas to calculate the total floral resource per site.

3) **Agricultural chemical loadings** were collated by conducting questionnaire surveys of all landowners and land managers for land within the field sites. The response rate to these questionnaires was approximately 50%, corresponding to an area of approximately 30% of the field sites. It was not possible therefore to validate the entire metric. Instead, direct comparison was made between the estimated and measured values for the fields covered by the questionnaire responses. Field values were summed for each tetrad.
4) **Managed honey bee density** was assessed by surveying each site using pan-trapping and field observations along predetermined transects. The total number of honey bees caught or observed was summed for each site to provide an index of honey bee density.

Results

Region and site selection

The six focal regions and 96 survey sites chosen by the protocol are shown in Fig. 1. From southeast to northwest, the focal regions covered parts of 1) Cambridgeshire, Suffolk and Norfolk, 2) Wiltshire and Gloucestershire, 3) Staffordshire, Cheshire, Shropshire and North East Wales, 4) North Yorkshire and Cumbria, 5) Ayrshire, Lanarkshire and East Renfrewshire, and 6) Inverness-shire.

Survey sites were generally well-selected in line with the criteria of the protocol, with some exceptions. Fig. 2 illustrates the contrasting values of the four estimated metrics for the

Cambridgeshire/Suffolk region as an example. The goal of the selection protocol was to effectively ensure that the bars were as high as possible for the "high" values (positive values in Fig. 2) and as low as possible for the "low" values (negative values in Fig. 2). In some cases sites could not be located at optimal locations due to access restrictions or were sub-optimal because of insufficiencies in the selection data that only became evident after site visits had been conducted. The wide range of values for each metric allows comparisons between sites and regions using continuous variables, rather than categorical "high vs low" differences. Furthermore, although it was not a site selection criterion, the site selection protocol removed the inherent correlation between the estimated values of the four metrics both for all regions (Table 1), and within individual regions (Fig. S4 – S6).

Validation

In order to validate the site selection protocol, the observed values of each of the four metrics were tested against the predictions derived from national datasets using simple Spearman's rank correlation tests (R base package; R Core Team 2014). These correlations are shown graphically in Fig. 3 and the coefficients are given in Table 2, together with results from linear mixed effects models using measured values as response variable, predicted values as explanatory variable, and region as random effect. Mixed models were performed using the package *nlme* in R 3.1.1 (R Core Team 2014). All four metrics showed significant positive relationships between the observed and predicted values. According to the correlation coefficients, the best predicted metric was habitat diversity, followed by insecticide loadings, floral resources, and honey bee density. However, it should be noted that the insecticide loading comparison omits tetrads for which questionnaire responses were not received, and tetrads for which measured insecticide could be assumed to be zero due to the absence of arable fields. If the latter are included, the Spearman's rank correlation coefficient is 0.57 ($p < 0.001$) but the slope of the regression is only 0.25 ($p<0.01$).

In terms of the correlations between metrics, there were significant relationships between the metrics for three out of the six pair-wise comparisons overall (Table 3), although the correlation coefficients were all below the commonly used threshold of 0.7 for including variables in the same analysis. Measured floral resources was significantly correlated with measured honey bee density (Spearman's $\rho = 0.31$, $S = 101440$, $p = 0.002$) and with measured insecticide loadings (Spearman's $\rho = -0.47$, $S = 89018$, $p <0.05$). In addition, measured honey bee density was strongly linked to measured insecticide loadings (Spearman's $\rho = 0.54$, $S = 58909$, $p <0.05$). However, for the individual regions (Fig. S7 – S9) the only significant correlations were for measured habitat diversity vs measured honey bee density in Inverness (Spearman's $\rho = 0.54$, $S = 312.7$, $p =0.03$; Fig. S7), measured insecticide loadings vs measured habitat diversity in Wiltshire (Spearman's $\rho = -0.92$, $S = 108$, $p <0.01$; Fig Sx) and for measured honey bee density vs measured insecticide loadings in Cambridgeshire (Spearman's $\rho = -0.65$, $S = 272$, $p = 0.04$; Fig. S9).

Discussion

The methodology described here served as the foundation for an ongoing nationwide, landscape-scale study into the links between land use and management practices and the recent documented declines in wild insect pollinators (Biesmeijer *et al.* 2006; Carvalheiro *et al.* 2013). The method aimed to utilise the existing variability in the British landscape to select sites that varied in four main gradients, while at the same time ensuring comparability between sites. The verification data has shown that not only is this possible, but that such a protocol can provide a useful site selection tool for a range of landscape scale studies.

Although estimations of the four metrics were made with some uncertainty, the low level of correlation between metrics at the site and regional scales suggest that the site selection method provides a suitable sample of sites for investigating significant factors that explain variation in pollinator populations.

Region and site selection

The selection of six representative regions each containing 16 focal sites was an efficient design for testing the effect of our four focal variables on insect pollinators across the whole of Britain, given constrained time and financial resources. As regions and sites in other studies are sometimes chosen because they are well known and have been used several times before in previous work, locations selected for study may not necessarily be the most appropriate or representative when viewed objectively. However, the selection methodology described here provides a protocol for ensuring objectivity when it is needed, and by being representative of a much wider area such as a country or biome, the generality of the results will also be broadened. The methodology allows the researcher to make informed, objective decisions about site selection with transparent and repeatable methods.

Each set of sixteen landscape-scale sites was chosen to vary in four potential drivers of pollinator decline as much as was possible within each region. While there was some uncertainty in estimating drivers, the set of sites selected was sufficiently dispersed in variable space to allow comparisons using continuous variables with good ranges. Randomly selected focal sites tend to cluster around mean values, providing relatively low resolving power for discerning the effects of landscape-scale drivers. Our original choice of what were modelled to be extreme values might be criticised for missing out these typical parameter values, but in practice the imprecise models combined with the inevitable regression towards the mean resulted in a wide exploration of parameter space of variables individually and in combination. This represents the first time that more than two independent variables of interest have been used to select field sites. In most studies to date, the main focus has been to select field sites that vary in one aspect such as land use intensity (Westphal, Steffan-Dewenter & Tscharntke 2003; Breitbach *et al.* 2012; Holzschuh, Dudenhoeffer & Tscharntke 2012; Williams, Regetz & Kremen 2012), or occasionally in two gradients such as landscape configuration and composition (Holzschuh, Steffan-Dewenter & Tscharntke 2010; Hopfenmueller, Steffan-Dewenter & Holzschuh 2014; Steckel *et al.* 2014). In contrast, this protocol demonstrates how a much more complex and broad study can be accommodated by using multiple variables to select sites objectively.

An additional, but untargeted benefit of the protocol is that the estimated variables were uncorrelated in the majority of pair-wise comparisons at both the national and regional scales, which is important for ensuring the independence of the variables as predictors and avoiding problems of collinearity in subsequent analyses. Focal variables can often be correlated in nature (e.g. intensive agricultural landscapes have both low habitat diversity and high insecticide use), so disrupting these correlations at the site selection stage is a further advantage of this methodology. Although at the national scale there were significant correlations between some verified metrics due to varying accuracy in metric estimates, they remained weaker than in the unfiltered dataset, and the lack of correlation was maintained within most regions. This suggests that improvements to the metric estimates could help achieve this at the national scale.

Site validation

The estimates of the four metrics varied in their accuracy quite widely. The most accurate was the habitat diversity metric which was based on the proportion of habitat covers calculated from remote sensing data. The high accuracy of this metric is not surprising as the estimates required the fewest steps in making the calculations, and verification was relatively straightforward. Even where the precise nature of land cover was misclassified on LCM2007, the spatial configuration of habitats as determined on the ground, and thus the Shannon index value, was generally quite close to our estimates from the LCM data. The level of accuracy is also similar to previous verification efforts (Morton *et al.* 2011). Nevertheless, validation could have been further improved by more extensive *in situ* habitat classification before any of the metrics were estimated.

The insecticide metric was also relatively well predicted when only considering those fields for which questionnaire responses were received. However, this result masks the large number of tetrads (especially in the North) for which large positive insecticide loadings were predicted when no arable fields were found on the ground. Although insecticides are applied on non-arable fields, the extent of application is unlikely to warrant a “high” insecticide loading value. Calculating this estimate required the use of the area cover of crops and differences in arable cover may be caused by estimating crop areas from disaggregation of holding level records or by changes in the crop areas between the 2010 census and 2012/13 survey years due to normal crop rotation. Recently reseeded pastures replacing arable fields were a particular case which resulted in original moderate insecticide use estimates for what were in fact entirely grassland (and largely insecticide free) landscapes in some northern regions. Suitable verification of this metric was further hampered by a lack of response to questionnaires by land managers; more intensive personal contact or the adoption of Fera Pesticide Usage Survey protocols in future might increase response rates.

The floral resource metric proved to have relatively low accuracy for a number of reasons related to the data available for making estimates: 1) some habitat cover estimates were incorrect due to misclassification in LCM2007 as described above, 2) floral reward data were only available for relatively few species at the time of site selection, 3) estimates of species cover per habitat were based on regional averages per broad habitat and so were not sensitive to within-region variation, 4) mean nectar availability reported in databases does not capture the high variability observed in the field due to site differences in climate, soil and nectar consumption. The improvement of factor 1) by validating habitat covers *in situ*, factor 2) by developing a database of floral rewards (Baude *et al.* 2016) and factors 3) and 4) via *in situ* surveying of flower species cover per habitat on each site, inevitably led to some widely differing values of site-level floral resource availability. Furthermore, the factors varied widely between the regions (e.g., habitat covers were poorly estimated in Yorkshire and Cumbria, but well estimated in Inverness-shire), and this resulted in variable regional accuracy of floral resource metrics (Fig S10).

The honey bee density metric was the least well verified of the four drivers partly because the methods used to count the number of honey bees visiting sites proved to be unsuitable. As honey bees are social foragers, using scouts to alert workers to rich floral resource patches, the use of pan trapping to sample them is extremely inefficient (Westphal *et al.* 2008). Further, attempts to observe honey bees on the wing or foraging along transects suffered from a lack of available survey time: only 3 full days per season per site were used, often in poor weather conditions as pan trapping was prioritised on good weather days (which were rare during those two summers). Where data are available, they show a good relationship with the estimated density. However, such is the noise in the data and the high presence of zeros that subsequent analysis will need to use the original estimated values as an explanatory variable. Better estimates of honey bee numbers would require either greater investment in survey time or an alternative method such as the use of baited traps or estimating the number of hives present through, for example, surveys of farmers and beekeepers. As a result of these problems, we are not able to verify the accuracy of the honey bee population density estimation technique.

Overall evaluation and implications

The aims of this site selection methodology were to improve on previous landscape-scale natural experimental designs by i) increasing objectivity, ii) enabling analysis of the interacting and partial contributions of several key factors by ensuring their crossing, replication and randomisation, and iii) enhance the ability to generalise results to the wider landscape. This has been achieved by i) selecting from the full set of candidate sites using objective criteria, ii) selecting sites based on their values of multiple focal variables, and iii) hierarchically stratified sampling across the entire country.

This study has shown that it is possible to use national datasets to derive credible and objective sets of study sites that cover multiple environmental gradients, without bias from researcher's personal knowledge of landscapes in the site selection. This represents a step forward in landscape-scale observational studies of land use and ecological processes, as previous studies have only accounted for one or two variables. The implications of this methodological development are important for landscape ecology and national scale monitoring programmes in any region or country with sufficient data, with a network of well-chosen sampling sites being a vital tenet of a well-designed national monitoring scheme.

The generality of results arising from analyses of pollinator population data against the four gradients has likely been enhanced by the protocol. The representative regions and sites provide generality of results across a wide range of geographical and management scenarios not normally seen in landscape scale studies, and the selected sites could be used as the basis for studies addressing other questions about land use impacts on environmental and ecological processes.

Acknowledgements

We are especially grateful to our network of farmers, landowners and land managers who provided access to their land to conduct ground truthing and our field assistants for conducting the field work: Nicole Dunn, Hayley Wiswell, Ewan Munro, Katy Donald, Jessica Heikkinen, Katherine White, Clare Pemberton, Paul Webb, Mark Tilzey, Sara Iversen, Robin Curtis, Paul Hill, Sam Bacon, Paul Wilson, Bex Cartwright, John Fitzgerald, Patrick Hancock, Mel Stone, Robert Day, James McGill, Tracie Evans. Stéphane Pietravalle, Kate Somerwill, and Mette Kusk Gillespie are thanked for assistance with the protocol design. This research was supported by the UK Insect Pollinator Initiative project "Agriland: Linking agriculture and land use change to pollinator populations", funded under the Living with Environmental change programme, a collaboration between Biotechnology and Biological Sciences Research Council (BBSRC), the Wellcome Trust, Scottish Government, Department of Environment, Food and Rural Affairs (DEFRA) and Natural Environment Research Council (NERC): grant BB/H014934/1 (www.agriland.leeds.ac.uk).

Data Accessibility:

References

Baude, M., Kunin, W.E., Boatman, N.D., Conyers, S., Davies, N., Gillespie, M.A.K., Morton, R.D., Smart, S.M. & Memmott, J. (2016) Historical nectar assessment reveals the fall and rise of floral resources in Britain. *Nature,* **530,** 85-+.

Beekman, M. & Ratnieks, F.L.W. (2000) Long-range foraging by the honey-bee, Apis mellifera L. *Functional Ecology,* **14,** 490-496.

Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science,* **313,** 351-354.

Bowers, M.A. & Dooley, J.L. (1999) A controlled, hierarchical study of habitat fragmentation: responses at the individual, patch, and landscape scale. *Landscape Ecology,* **14,** 381-389.

Breitbach, N., Tillmann, S., Schleuning, M., Gruenewald, C., Laube, I., Steffan-Dewenter, I. & Boehning-Gaese, K. (2012) Influence of habitat complexity and landscape configuration on pollination and seed-dispersal interactions of wild cherry trees. *Oecologia,* **168,** 425-437.

Brittain, C.A., Vighi, M., Bommarco, R., Settele, J. & Potts, S.G. (2010) Impacts of a pesticide on pollinator species richness at different spatial scales. *Basic and Applied Ecology,* **11,** 106-115.

Bunce, R.G.H., Barr, C.J., Clarke, R.T., Howard, D.C. & Lane, A.M.J. (1996) ITE Merlewood Land Classification of Great Britain. *Journal of Biogeography,* **23,** 625-634.

Carey, P.D., Wallis, S., Chamberlain, P.M., Cooper, A., Emmett, B.A., Maskell, L.C., McCann, T., Murphy, J., Norton, L.R., Reynolds, B., Scott, W.A., Simpson, I.C., Smart, S.M. & Ullyett, J.M. (2008) Countryside Survey: UK Results from 2007. Centre for Ecology & Hydrology.

Carvalheiro, L.G., Kunin, W.E., Keil, P., Aguirre-Gutierrez, J., Ellis, W.N., Fox, R., Groom, Q., Hennekens, S., Van Landuyt, W., Maes, D., Van de Meutter, F., Michez, D., Rasmont, P., Ode, B., Potts, S.G., Reemer, M., Roberts, S.P.M., Schaminee, J., WallisDeVries, M.F. & Biesmeijer, J.C. (2013) Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants. *Ecology Letters,* **16,** 870-878.

Cumming, G.S., Olsson, P., Chapin, F.S., III & Holling, C.S. (2013) Resilience, experimentation, and scale mismatches in social-ecological landscapes. *Landscape Ecology,* **28,** 1139-1150.

Diamond, J.M. (1983) ECOLOGY - LABORATORY, FIELD AND NATURAL EXPERIMENTS. *Nature,* **304,** 586-587.

Dilts, T.E., Yang, J. & Weisberg, P.J. (2010) The Landscape Similarity Toolbox: new tools for optimizing the location of control sites in experimental studies. *Ecography,* **33,** 1097-1101.

Elbgami, T., Kunin, W.E., Hughes, W.O.H. & Biesmeijer, J.C. (2014) The effect of proximity to a honeybee apiary on bumblebee colony fitness, development, and performance. *Apidologie,* **45,** 504-513.

Evison, S.E.F., Roberts, K.E., Laurenson, L., Pietravalle, S., Hui, J., Biesmeijer, J.C., Smith, J.E., Budge, G. & Hughes, W.O.H. (2012) Pervasiveness of Parasites in Pollinators. *Plos One,* **7**.

Gabriel, D. & Tscharntke, T. (2007) Insect pollinated plants benefit from organic farming. *Agriculture Ecosystems & Environment,* **118,** 43-48.

Goulson, D. & Sparrow, K. (2009) Evidence for competition between honeybees and bumblebees; effects on bumblebee worker size. *Journal of Insect Conservation,* **13,** 177-181.

Hargrove, W.W. & Pickering, J. (1992) PSEUDOREPLICATION - A SINE-QUA-NON FOR REGIONAL ECOLOGY. *Landscape Ecology,* **6,** 251-258.

HilleRisLambers, J., Ettinger, A.K., Ford, K.R., Haak, D.C., Horwith, M., Miner, B.E., Rogers, H.S., Sheldon, K.S., Tewksbury, J.J., Waters, S.M. & Yang, S. (2013) Accidental experiments: ecological and evolutionary insights and opportunities derived from global change. *Oikos,* **122,** 1649-1661.

Hobbs, N.T. (2003) Challenges and opportunities in integrating ecological knowledge across scales. *Forest Ecology and Management,* **181,** 223-238.

Holzschuh, A., Dudenhoeffer, J.-H. & Tscharntke, T. (2012) Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. *Biological Conservation,* **153,** 101-107.

Holzschuh, A., Steffan-Dewenter, I. & Tscharntke, T. (2010) How do landscape composition and configuration, organic farming and fallow strips affect the diversity of bees, wasps and their parasitoids? *Journal of Animal Ecology,* **79,** 491-500.

Hopfenmueller, S., Steffan-Dewenter, I. & Holzschuh, A. (2014) Trait-Specific Responses of Wild Bee Communities to Landscape Composition, Configuration and Local Factors. *Plos One,* **9**.

Mitchell, M.S., Rutzmoser, S.H., Wigley, T.B., Loehle, C., Gerwin, J.A., Keyser, P.D., Lancia, R.A., Perry, R.W., Reynolds, C.J., Thill, R.E., Weih, R., White, D. & Wood, P.B. (2006) Relationships between avian richness and landscape structure at multiple scales using multiple landscapes. *Forest Ecology and Management,* **221,** 155-169.

Morton, D., Rowland, C., Wood, C., Meek, L., Marston, C., Smith, G., Wadsworth, R. & Simpson, I. (2011) Final Report for LCM2007-the new UK land cover map. *Countryside Survey*. Centre for Ecology & Hydrology.

Potts, S.G., Vulliamy, B., Dafni, A., Ne'eman, G. & Willmer, P. (2003) Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology,* **84,** 2628-2642.

R Core Team (2014) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramankutty, N., Evan, A.T., Monfreda, C. & Foley, J.A. (2008) Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. *Global Biogeochemical Cycles,* **22**.

Rortais, A., Arnold, G., Halm, M.P. & Touffet-Briens, F. (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie,* **36,** 71-83.

Roux, E., Gaborit, P., Romana, C.A., Girod, R., Dessay, N. & Dusfour, I. (2013) Objective sampling design in a highly heterogeneous landscape - characterizing environmental determinants of malaria vector distribution in French Guiana, in the Amazonian region. *Bmc Ecology,* **13**.

Rundlof, M., Andersson, G.K.S., Bommarco, R., Fries, I., Hederstrom, V., Herbertsson, L., Jonsson, O., Klatt, B.K., Pedersen, T.R., Yourstone, J. & Smith, H.G. (2015) Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature,* **521,** 77-U162.

Sagarin, R. & Pauchard, A. (2010) Observational approaches in ecology open new ground in a changing world. *Frontiers in Ecology and the Environment,* **8,** 379-386.

Shackelford, G., Steward, P.R., Benton, T.G., Kunin, W.E., Potts, S.G., Biesmeijer, J.C. & Sait, S.M. (2013) Comparison of pollinators and natural enemies: a meta-analysis of landscape and local effects on abundance and richness in crops. *Biological Reviews,* **88,** 1002-1021.

Smart, S.M., Henrys, P.A., Purse, B.V., Murphy, J.M., Bailey, M.J. & Marrs, R.H. (2012) Clarity or confusion? - Problems in attributing large-scale ecological changes to anthropogenic drivers. *Ecological Indicators,* **20,** 51-56.

Steckel, J., Westphal, C., Peters, M.K., Bellach, M., Rothenwoehrer, C., Erasmi, S., Scherber, C., Tscharntke, T. & Steffan-Dewenter, I. (2014) Landscape composition and configuration differently affect trap-nesting bees, wasps and their antagonists. *Biological Conservation,* **172,** 56-64.

Stow, C.A., Carpenter, S.R., Webster, K.E. & Frost, T.M. (1998) Long-term environmental monitoring: Some perspectives from lakes. *Ecological Applications,* **8,** 269-276.

Tanentzap, A.J., Lamb, A., Walker, S. & Farmer, A. (2015) Resolving Conflicts between Agriculture and the Natural Environment. *Plos Biology,* **13**.

Vanbergen, A.J., Baude, M., Biesmeijer, J.C., Britton, N.F., Brown, M.J.F., Brown, M., Bryden, J., Budge, G.E., Bull, J.C., Carvell, C., Challinor, A.J., Connolly, C.N., Evans, D.J., Feil, E.J., Garratt, M.P., Greco, M.K., Heard, M.S., Jansen, V.A.A., Keeling, M.J., Kunis, W.E., Marris, G.C., Memmott, J., Murray, J.T., Nicolson, S.W., Osborne, J.L., Paxton, R.J., Pirk, C.W.W., Polce, C., Potts, S.G., Priest, N.K., Raine, N.E., Roberts, S., Ryabov, E.V., Shafir, S., Shirley, M.D.F., Simpson, S.J., Stevenson, P.C., Stone, G.N., Termansen, M., Wright, G.A. & Insect Pollinators, I. (2013) Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment,* **11,** 251-259.

Waddington, K.D., Visscher, P.K., Herbert, T.J. & Richter, M.R. (1994) COMPARISONS OF FORAGER DISTRIBUTIONS FROM MATCHED HONEY-BEE COLONIES IN SUBURBAN ENVIRONMENTS. *Behavioral Ecology and Sociobiology,* **35,** 423-429.

Westphal, C., Bommarco, R., Carre, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S.G., Roberts, S.P.M., Szentgyorgyi, H., Tscheulin, T., Vaissiere, B.E., Woyciechowski, M., Biesmeijer, J.C., Kunin, W.E., Settele, J. & Steffan-Dewenter, I. (2008) MEASURING BEE DIVERSITY IN DIFFERENT EUROPEAN HABITATS AND BIOGEOGRAPHICAL REGIONS. *Ecological Monographs,* **78,** 653-671.

Westphal, C., Steffan-Dewenter, I. & Tscharntke, T. (2003) Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters,* **6,** 961-965.

Westphal, C., Steffan-Dewenter, I. & Tscharntke, T. (2006) Bumblebees experience landscapes at different spatial scales: possible implications for coexistence. *Oecologia,* **149,** 289-300.

Williams, N.M., Regetz, J. & Kremen, C. (2012) Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. *Ecology,* **93,** 1049-1058.

Wu, J.G. & Hobbs, R. (2002) Key issues and research priorities in landscape ecology: An idiosyncratic synthesis. *Landscape Ecology,* **17,** 355-365.

Tables

Table 1: Spearman correlation coefficients for the four estimated metrics (Box-Cox transformed Z-scores) for all six study regions. Coefficients are calculated for all possible sites within all regions (n = 12,718 sites) and the sites selected for study (n = 96). Asterisks denote significant correlations ($p<0.001$). Partial correlation coefficients were calculated controlling for Region, but are not shown as they were not different from the coefficients below.

	Habitat diversity		**Floral resources**		**Insecticide loadings**	
	All possible sites	Selected sites	All possible sites	Selected sites	All possible sites	Selected sites
Floral resources	0.14*	0.11	-	-	-	-
Insecticide loadings	-0.28*	-0.16	-0.20*	-0.16	-	-
Honey bee density	0.10*	0.10	-0.15*	-0.08	0.24*	0.11

Table 2: Spearman's rank correlation and partial correlation coefficients (controlling for Region), and parameters of linear mixed models (Region as random effect) for the estimated versus measured metrics in all regions. The data are Z-scores: box-cox transformed and zero centred. "Mean floral resources" is the total amount of floral resources averaged over the two years of field sampling. Asterisks indicate significant correlations: *** = p<0.001, ** = p<0.01, * = p<0.05

	Overall correlation	**Partial correlation**	**Slope**	**Intercept**	**P**
Habitat diversity	0.77***	0.77***	0.56	-0.05	<0.001
Mean floral resources	0.28**	0.29**	0.20	-0.03	0.005
Insecticide loadings	0.67**	0.60**	0.67	-0.01	0.001
Honey bee density	0.22*	0.21*	0.16	0.03	0.002

Table 3: Spearman's rank correlation and partial correlation (controlling for region) coefficients for the four measured metrics (Box-Cox transformed Z-scores) for all six study regions. Asterisks indicate significant correlations (* = p<0.05, ** = p<0.01).

	Habitat diversity	**Floral resources**	**Insecticide loadings**
All regions			
Floral resources	0.18		
Insecticide loadings	-0.47*	0.10	
Honey bee density	-0.04	0.31**	-0.54*
All regions (partial correlation)			
Floral resources	0.16		
Insecticide loadings	NA	NA	
Honey bee density	-0.05	0.29**	NA

Figures

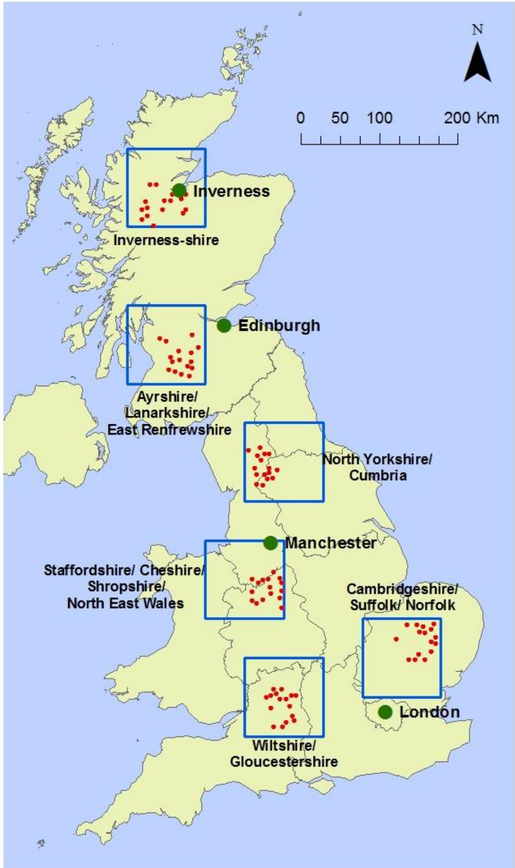

Fig. 1: The extent of the six 100 km^2 regions chosen by the region selection protocol (blue squares), and the 96 field sites (sixteen 2 x 2 km^2 sites per region) chosen by the site selection protocol (red circles). (Service Layer Credit: OS data; Crown copyright and database right 2015)

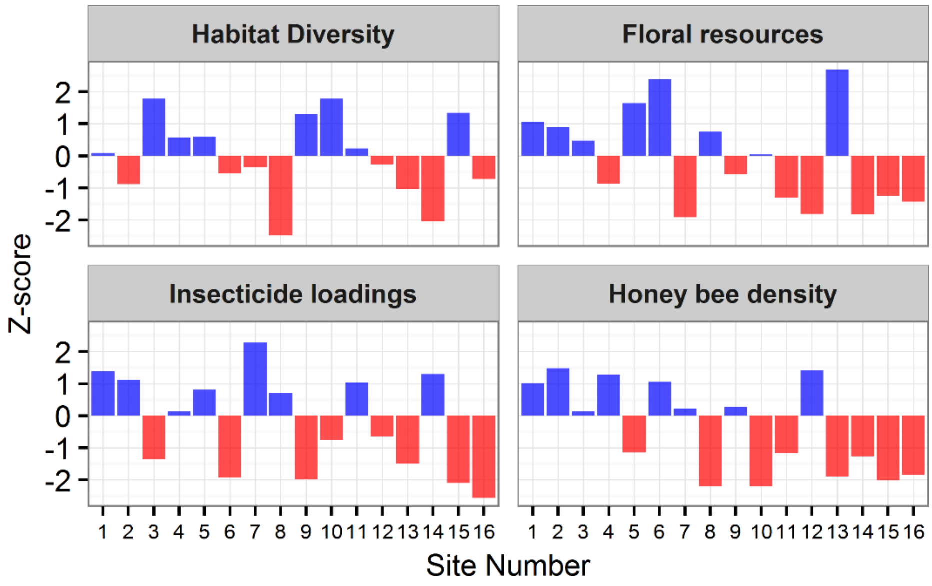

Fig. 2: The estimated Z-scores (Box-Cox transformed and zero centred data) of the four metrics for the final 16 sites of the Cambridgeshire/Suffolk region, shown here as an example. The blue bars are Z-scores above 0, i.e., the site has a “high” score for that metric; the red bars are negative Z-scores, i.e., the site has a “low” score for that metric. The 16 sites represent every combination of high and low values of the four metrics, e.g., site 1 has high values of all four metrics, site 2 has a low value only for habitat diversity, and so on. The data for the remaining regions can be found in Fig. S3

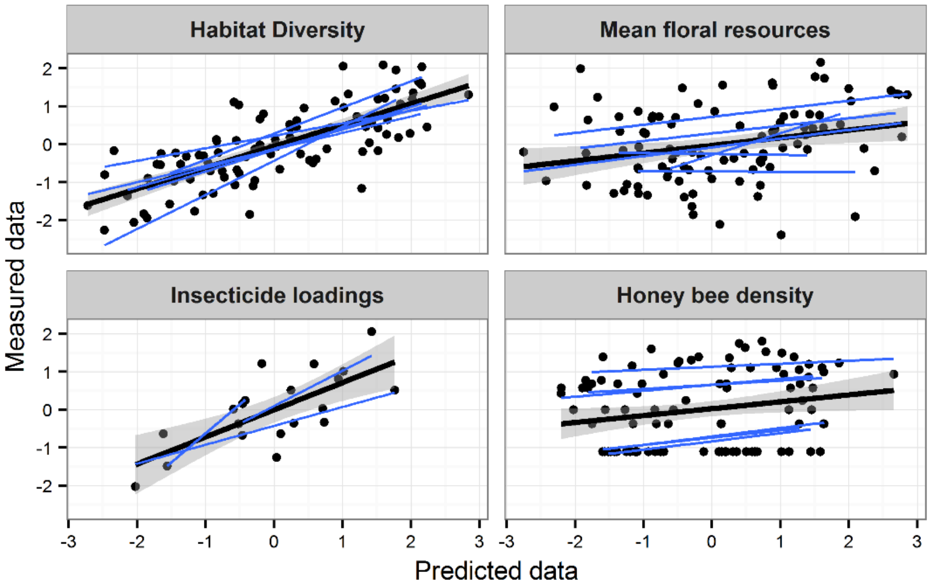

Fig. 3: "Ground-truthing" of the four key metrics. The data are Z-scores: box-cox transformed and 0 centred. The straight bold line represents the linear regression line for all regions and the shaded area represents 95% confidence intervals. The blue lines are mixed effect regression lines for each of the six regions with "region" as a random effect, displayed here to demonstrate the variation in prediction accuracy between regions. "Mean floral resources" is the total amount of floral resources averaged over the two years of field sampling. Regional graphs are shown in Fig. S10.